

Appendix 2B-2: Status Report on the Effect of Water Quantity and Quality on Methylmercury Production

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THE EFFECT OF THE SULFUR CYCLE ON METHYLMERCURY PRODUCTION

In 1994, a consortium of agencies led by the USGS began a multi-investigator study of the factors contributing to the high levels of Hg in Everglades biota, the Aquatic Cycling of Mercury in the Everglades (ACME) project. The project has focused on the processes that lead to from Hg deposition to MeHg formation and bioaccumulation. Initial hypotheses, that methylmercury (MeHg) production is the key control on MeHg bioaccumulation, and that MeHg production is generally favored in wetlands, were born out (Hurley et al., 1998; Krabbenhoft et al 1998; Gilmour et al 1998a,b; Marvin-DiPasquale et al., 1998; 2000). Among the addition findings, the ACME project identified the important influence of sulfur inputs to the Everglades on Hg methylation within the ecosystem (Gilmour et al., 1998a,b; Cleckner et al., 1999; Bates et al., in press; Orem et al., in review).

Surface water sulfate concentrations in northern WCA 2A average roughly 50 mg/L, more than a hundred to a thousand times higher than sulfate levels in the ENP and in southern WCA-3A (Orem et al., in review; Gilmour et al., 1998b; EPA/REMAP). Sulfur stable isotope data and the depth-based historical record suggest that sulfur concentrations in Everglades peats are elevated above historical ambient concentrations over most of the ecosystem (Bates et al., 2001, and in press). Sulfur may enter the Everglades from a number of potential sources including canal water discharge, rainfall and groundwater. One important source is sulfur amendments to agricultural fields in the EAA, used to enhance phosphorus uptake by sugarcane and other crops through modification of soil acidity. This sulfur has a stable sulfur isotopic signal distinct from most other sources. This signal has been observed in EAA soils, and in the sulfate in canal water draining the EAA. The high levels of sulfate observed in canal water in the EAA and in areas of the Everglades receiving canal water discharge is thought to arise from runoff of this agricultural sulfur (Bates et al., 2001, and in press; Orem et al., in review).

Sulfur enrichment in the Everglades is a stressor with specific impacts on MeHg production, as well as wider impacts on the ecosystem. In the Everglades, MeHg is generally produced in the top few cm of unconsolidated detritus and consolidated peat soil (Gilmour et al., 1998b; Cleckner et al., 1999), by obligately anaerobic bacteria, primarily sulfate-reducing bacteria (SRB) (Compeau and Bartha, 1985; Gilmour et al., 1991; Gilmour et al., 1998a). Sulfate-reducing bacteria degrade organic matter in sediments, using sulfate as the electron acceptor. The end

product of microbial sulfate-reduction activity is sulfide. Anaerobic microbial processes, including sulfate reduction, are key components of microbial organic carbon decomposition in Everglades peats and surficial flocs. The primary factors that control the metabolic activity of SRB are temperature, dissolved oxygen (DO), organic carbon supply, and sulfate supply.

Aside from its impact on MeHg production in the Everglades, sulfur contamination of the ecosystem may have other deleterious effects. Sulfur appears to be a major control on redox conditions in the sediments. Areas of the Everglades contaminated with sulfur from canal water discharge (e.g. WCA 2A and northern WCA 3A) have significantly more negative (more reducing) redox conditions compared to sites with background levels of sulfur (e.g. WCA 1, southern WCA 3A, and much of ENP; Orem et al., in prep). The lower redox conditions in the sediment may negatively affect rooted macrophytes not adapted to these highly reducing conditions by reducing oxygen flow to the roots. Tree islands may also be negatively impacted by the more reducing soil conditions resulting from sulfur contamination. Further study is needed to evaluate the extent of these impacts.

Microbial sulfate reduction appears to be the most important mechanism for reduced S storage in Everglades peat. Stable isotope signatures show that the majority of reduced S stored in Everglades sediments, at both eutrophic and more pristine sites, arises from dissimilatory sulfate reduction rather than assimilation by plants (Bates et al., 1998 and 2001; Orem et al., in review; Kendall et al., in prep). Sulfate reduction was readily measured in surface sediments across the Everglades using isotopic tracers (Gilmour et al., 1998; Heyes et al., 1998).

Net MeHg accumulation in surface sediments represents the balance of microbial methylation and photochemical and microbial demethylation processes. Because Everglades food webs are generally benthic, the concentration of MeHg in surface sediments is an excellent predictor of MeHg in biota (Krabbenhof et al., 1998). Instantaneous methylation rates are also good predictors of MeHg concentrations in surface sediments (Gilmour et al., 2001; Heyes et al., 2001). Methylmercury concentrations in the top 0 to 4 cm of sediment are now used as primary indicators of MeHg production in the Everglades.

Because SRB are important Hg methylators, sulfate influences methylmercury production. However, the interaction between the Hg and S cycles is complex. While sulfur has a large impact on MeHg production, the magnitude and even direction of the impact varies with the sulfate and sulfide concentration. Sulfate stimulates the activity of Hg-methylating microorganisms, and can therefore increase MeHg concentrations (Gilmour et al., 1991; Watras et al., 1994; Branfireun et al., 1999; King et al., 1999; 2000). However, the bioavailability of Hg to methylating bacteria also affects MeHg production rates by bacteria. Sulfide has a very strong impact on the bioavailability of inorganic mercury to methylating bacteria (Compeau et al., 1983; Craig et al., 1983; Choi et al., 1994). There is an optimal sulfide concentration for Hg uptake, which is quite low. Above that concentration sulfide inhibits bioavailability. These two forms of sulfur, sulfate and sulfide, whose concentrations are often correlated across the Everglades (Orem et al in prep; Gilmour et al., 1998b), have opposing effects on Hg methylation. Understanding the quantitative relationships between sulfate stimulation of methylation and sulfide inhibition of methylation across the large sulfur gradient that exists in the Everglades is necessary to understand how sulfur in agricultural runoff affects MeHg in Everglades fish.

All available data point to a MeHg production maximum in the central Everglades. Large mouth bass (Lange, pers. comm. 2002) and Gambusia (Krabbenhof et al., 1998, in prep; EPA/REMAP) concentrations are highest in WCA-3A. ACME data show maxima in both MeHg concentration and production in surface sediments in central WCA-3A (Gilmour et al., 1998b; in prep.). Based on the ACME data set, and geochemical modeling, the ACME team proposed the

following model for control of MeHg production by sulfur in the Everglades: 1) high concentrations of sulfide inhibit MeHg production in the northern part of the ecosystem, 2) intermediate sulfate (~50-100 μM) and low sulfide (<10 μM) concentrations in the central part of the system (e.g. WCA 3A) are optimal for methylation, and 3) sulfate concentrations in the most pristine areas (<25 μM) are sub-optimal for sulfate-reduction and MeHg production. This scenario implies that the excess load of sulfur to the Everglades minimizes MeHg production in WCA 2A, but stimulates MeHg production in WCAs 2B and 3A.

ACME field data, and research on the impact of sulfide on methylation, strongly support the hypothesis that sulfide inhibits methylation in the northern Everglades.

The ACME data set consists of triennial samples collected in the period March 1995-July 1999 from 10 sites along a generally north-south transect through the Everglades. In these data, a strong inverse relationship was observed between the sulfide concentration in sediment pore waters and the concentration of methylmercury in surface sediments (Gilmour et al., 1998a,b; in prep). Of the many variables examined, pore water sulfide was the single best predictor of sediment MeHg concentrations.

During the ACME study, the chemical speciation of Hg in sulfidic waters was studied, and mechanism of sulfide inhibition of methylation was examined. Substantial changes in existing models for this process were derived, and these are leading to better modeling of Hg methylation in ecosystems (TetraTech, 2002). Benoit et al., proposed that sulfide controls Hg bioavailability to SRB by controlling the concentration of neutral dissolved Hg species that diffuse across cell membranes. This model is different from older models in that 1) sulfide inhibition of Hg methylation is not simply caused by decreased concentration of dissolved inorganic Hg (Hg_D), due to precipitation of $\text{HgS}_\text{(s)}$, 2) the model includes the existence of small dissolved neutral Hg-S complexes and 3) the model assumes Hg uptake into cells via passive diffusion, rather than active uptake of divalent metal cations. The existence of neutral Hg-sulfide complexes, probably HgS^0 , is supported by both geochemical models (Benoit et al., 1999a) and experimental partitioning studies (Benoit et al., 1999b). Benoit's model for Hg partitioning and speciation in sulfidic pore waters shows a strong relationship between predicted HgS^0 concentration in the dissolved phase and MeHg concentration in bulk sediment in two ecosystems, the Everglades, and a temperate estuary.

The uptake of HgS^0 was also demonstrated in pure cultures of the methylating SRB *Desulfobulbus propionicus* (Benoit et al., 2001a). Methylmercury production by cultures was not related to the absolute solid-phase concentration of Hg in the ores, and it was only weakly related to the dissolved inorganic Hg concentration in the medium. However, MeHg production was linearly related to the calculated concentration of the dominant neutral complex in solution, HgS^0 . Furthermore, the diffusive membrane permeability of HgS^0 , as estimated from its octanol-water partitioning coefficient, was found to be sufficient to support MeHg production by cells (Benoit et al., 2001b). Slightly large neutral Hg-polysulfide complexes may also be found in sediment pore waters (Jay et al., 2000), however, these appear to be too large to diffuse across cell membranes at a rate sufficient to support significant Hg methylation (Jay et al., in prep.).

The idea that sulfate stimulates methylation in much of the central Everglades is less strongly supported by correlations in the ACME field data, possibly because of lower data density in the central and southern ecosystem. Sulfate does stimulate MeHg production when added to sediment cores taken from sites with lower (<5 mg/L) sulfate concentrations (Bell et al., 1999; Gilmour et al., 2000; Gilmour et al., in prep). In these studies, methylation rates increased linearly with sulfate addition up to a point and then decreased with increasing pore water sulfide concentrations. The optimal sulfate concentration appears to vary somewhat among Everglades

regions, because of how sediment chemistry affects the solubility of sulfide produced by SRB. These experimental studies support the idea that the very low sulfate concentrations found in Taylor Slough and central LNWR limit the activity of SRB and therefore limit methylation rates. Sulfate concentrations in the freshwater marsh within ENP, and in central LNWR probably best reflect historical sulfate concentrations, which would have been derived mainly from rain in the freshwater Everglades.

In order to improve our understanding of the relationships between sulfate and MeHg in the Everglades, sulfate-addition mesocosm studies were initiated by ACME in late 2001. The objective of these studies is to specifically test for sulfate stimulation of methylation and sulfate-reduction at “lower” sulfate sites in the Everglades. Mesocosms are being amended at two sites, one in central LNWR and one in central WCA-3A. Sulfate concentrations of 5, 10 and 20 mg/L are being added to the mesocosms over the course of two months. The effect of sulfate on methylation of both existing Hg pools and newly deposited Hg will be tested, using Hg stable isotope additions. Additionally, the dose-response to Hg additions is being tested, as well as the influence of dissolved organic carbon on Hg cycling and methylation.

Dissolved organic carbon (DOC) carbon directly affects the complexation of Hg in pore waters, and also affect the solubility of sulfide and of Hg-S complexes. Cinnabar precipitation can be retarded or reversed in the presence of certain fractions of Everglades DOC (Ravichandran et al., 1998). Understanding the relative strength of DOC and sulfide as ligands for Hg is important to modeling Hg behavior, including methylation. Recently, Benoit et al. (2001) characterized conditional stability constants for Hg with two DOC isolates from WCA 2A. These are similar to previously published stability constants for Hg binding to low molecular weight thiols, and show a pH-dependency that is consistent with models of Hg complexation with thiol groups as the dominant Hg binding sites in DOM. These experiments demonstrate that the DOM isolates are stronger ligands for Hg than chloride ion or EDTA. Speciation calculations indicate that at the DOM concentrations frequently measured in Everglades, 20 to 40 μM , significant complexation of Hg by DOM would be expected in aerobic (sulfide-free) surface waters. However, even nanomolar sulfide concentrations would be expected to out-compete DOM for Hg binding at any realistic ($\mu\text{l mM}$) DOM concentration (Benoit et al., 1999a). These results are consistent with the dominance of Hg-sulfide complexes in pore waters in the Everglades. However, it should be noted that interactions other than simple ligand exchange may influence the reactivity of natural DOM toward Hg in sulfidic environments. For example, Ravichandran et al. (1998, 1999) showed that F1-HPoA enhanced dissolution and inhibited precipitation of $\text{HgS}_{(s)}$ to a greater degree (by an order of magnitude) than either 2BS-HPiA or thioglycolate, although these ligands appear to have similar stability constants. Furthermore, a chemical equilibrium approach does not address how the kinetics of Hg-S-DOM interactions may affect the complexation of Hg in sediments with dynamic sulfide and DOM pools.

The influence of sulfate and sulfide on demethylation is less well characterized. Demethylation may occur by microbial or photochemical pathways. Using ^{14}C -labelled methylmercury, microbial demethylation has been observed by obligate anaerobes via two different pathways (reductive or oxidative) leading to methane or carbon dioxide formation (Oremland et al., 1993; Marvin-DiPasquale and Oremland, 1998; Marvin-DiPasquale et al., 2000; Marvin-DiPasquale et al., 2001).

Methylmercury may also be decomposed to Hg(II) or Hg(0) by the action of sunlight in water or soil (Krabbenhoft et al., 2001; Krabbenhoft et al., 2002). The rate at which this occurs is determined by the concentration and character of dissolved organic carbon (DOC). DOC-complexed Hg(II) can be reduced to Hg(0) by the action of sunlight on DOC (Zhang and Lindberg, 2001a). Fe(III) stimulates Hg(0) production (Zhang et al., 2001). Once produced, Hg(0)

will evade directly to the atmosphere (Vandal et al., 1994; Lindberg et al., 1999; Lindberg and Zhang, 2000; Zhang and Lindberg, 2001b). Rooted macrophytes are also believed to pump Hg(0) from the root zone through stems to leaves and thence to the air (Lindberg et al., 1999). The influence of sulfur species on these processes is unknown.

In summary, sulfur exerts one of the strongest controls on net MeHg production in the Everglades. The inhibitory response of methylation to sulfide is fairly well understood mechanistically, and a simple equation relating pore water sulfide to sediment MeHg across the Everglades can be derived. Sulfide accumulating in sediment surface pore waters appears to limit MeHg production and accumulation in northern WCA-2A and in some of the STAs, especially ENR. It has been proposed that the high MeHg levels found across much of the central Everglades are driven by sulfate enrichment, through stimulation of the bacteria that produce MeHg. Small-scale experimental studies support this hypothesis, and larger scale mesocosm studies are underway.

THE EFFECT OF SOIL DRYOUT ON METHYLMERCURY PRODUCTION: APPLICATIONS TO THE STAS

Results of a joint USGS-District study of an Everglades dry out and burn that occurred in the spring of 1999 demonstrated a pronounced methylmercury production period when the dried/burned soils across the northern Everglades were rewetted (Krabbenhoft and Fink, 2000; Krabbenhoft et al., 2000; Krabbenhoft et al., 2001a,b). Understanding the causal factors that gave rise to the elevated levels of methylmercury in the dried/burned areas of the Everglades in 1999 has direct bearing on construction and management of STAs, and on how we view the effectiveness and efficacy of STA's in the overall Everglades restoration strategy.

The 1999 dry-burn study showed a pulse of methylmercury production in surface soils after inundation in June-July of 1999. The pulse was relatively short lived (days to a few weeks), with maximal sediment MeHg concentrations in July. However, the elevated pools of methylmercury in sediments and pore waters become part of the actively cycling mass in the environment. The pulse in methylmercury production was followed by substantial increases in methylmercury in *Gambusia* and in young-of-the-year sunfish and largemouth bass (Lange et al., 2000). Details of this study are provided in the 2000 ECR report, Appendix 7-8.

Krabbenhoft et al., concluded that the large pulse of MeHg production was most likely due to increased sulfate after drying and rewetting, and concomitant increases in the activity of methylating microorganisms that respire sulfate. The hydrated peat soils of the Everglades are normally anoxic within mm to cm of the soil surface, and most of the sulfur is stored in reduced forms (Bates et al., 1998; Orem et al., in review; Krabbenhoft and Fink, 2000; Fink, 2001). Drying and burning result in oxidation of soils, including oxidation of reduced sulfur stored in peat. Re-inundation of oxidized soils is usually accompanied by a "first-flush" release of oxidized nutrients (Newman and Pietro, 2000) and sulfur (Krabbenhoft et al., 2000). Following re-inundation, the peat soils very quickly return to an anoxic state (Kelly et al., 1997), and anaerobic sulfate-reduction is stimulated by the presence of the newly oxidized sulfur. It is important to note that in the 1999 study, the concentration of inorganic mercury in surface waters and in sediment pore waters was not elevated in the months following rewetting, suggesting soil oxidation did not release Hg, and that changes in Hg concentration are not driving the pulse of methylation. However, the study began a few weeks after inundation, and some initial response may have been missed. Sulfate, sulfide and methylmercury concentrations were significantly increased in dried or burned and rewetted soils. The relatively rapid decline from peak

methylmercury concentrations in pore water and soils was coincident with the rapid depletion of the elevated sulfate pool. This coincidence would suggest that the decline in methylmercury levels was a result of a depletion of sulfate. However, one cannot rule out two alternative hypotheses. The first is that methylation declined after a initial pulse because of the relatively rapid build up of sulfide after rewetting. The second is that the pulse in methylation after rewetting is driven by highly bioavailable Hg in rain.

Another gap in this puzzle is the source of mercury for the newly produced methylmercury. Inorganic mercury to feed the methylation process could be contributed by “old” mercury pools existing in the peat soils, from the rain and canal water that gives rise to the re-inundation, or both. Resolution of this important gap in our understanding is important for prescribing the proper management response in how to minimize future methylmercury production periods following drying and wetting cycles in the future. Mercury-addition studies in mesocosms conducted under Phase II of the ACME project should provide key insights into which pools of mercury are likely most responsible for fueling this response (Krabbenhof et al., 2001; Gilmour et al., 2001).

The effect of first flooding of dry land on inorganic mercury release, methylmercury production, and methylmercury bioaccumulation has also been observed numerous times during the construction of reservoirs (e.g. Bodaly et al., 1984; Scruton et al., 1994; Rodgers et al., 1995). This “reservoir effect” has been examined in detail in an experimentally flooded boreal peatland (St. Louis et al., 1994; St. Louis et al., 1996; Kelly et al., 1997; Paterson et al., 1998). In some settings, the production of new methylmercury and bioaccumulation is a short-term phenomenon, while in others it can persist for decades. Long-term elevated methylmercury production in reservoirs has generally been attributed to the slow degradation of organic matter in newly-flooded soils, fueling anaerobic microbial activity. Short-term responses in newly flooded wetlands may be fueled by oxidized sulfur, but these responses are less-well documented. In the extreme case, if sulfate and organic matter are present in substantial excess, sulfide may build up to the point that it inhibits methylmercury production (see above).

The ENR and the STAs have exhibited different behaviors with regard to methylmercury production and bioaccumulation. Throughout its lifetime, the ENR exhibited very low methylmercury production and correspondingly low concentrations in fish at all trophic levels (Cleckner et al., 1998; Lange et al., 1998, 1999; Loftus et al., 1998; Rumbold et al., 2000; Rawlik, 2001a; Rumbold et al., 2001). Sulfur levels in soils and sulfate in incoming waters are both very high in ENR, and it has been hypothesized that sulfide inhibition limited production of methylmercury there (Gilmour et al., 1998b). A pulse of methylmercury production and accumulation mosquitofish in STA-1W Cell 5 occurred within four weeks of flooding in May 1999. However, this cell relaxed back to ENR-like conditions in water and mosquitofish by January 2000. The mercury behavior of STA1W Cell 5 must be contrasted with that of STA-2 Cell 1. Within about eight weeks of reflooding, the concentration of unfiltered methylmercury rose in water to an unprecedented 4.8 ng/L, considered anomalously high relative to the Everglades Nutrient Removal (ENR) Project, which averaged about 0.1 ng/L. Water column concentrations oscillated up and down thereafter to concentrations as low as 0.1 ng/L until Cell 1 again began to dry out in mid-April 2001, when methylmercury concentrations rose to 4.2 ng/L. However, total mercury concentrations in mosquitofish increased throughout this same period and averaged about 350 µg/Kg wet weight when the last sample was collected in mid-March 2001. This value exceed the average value of about 200 µg/Kg wet weight at WCA-3A-15, the Everglades “hot spot.”

Results of the 1999 dry/burn study and of the ACME study suggest that these differences in STA behavior are most likely related to differences in wetting and drying regimes, differences in

historical sulfur accumulation in soils, or differences in sulfate concentrations in treatment water. Examination of the sulfur regime in the STAs is warranted, as are studies of studies of mercury and sulfur cycling in the STAs following rewetting. As noted in the 2000 ECR, CERP-related changes in drying and rewetting cycles may effect methylmercury production in the Everglades and these processes should be considered in risk-management strategies.

NEW FINDINGS IN 2001-2002

MODELING HG METHYLATION

The equations that model Hg methylation and demethylation, and the equations that determine Hg speciation in sediment pore waters are key routines in the E-MCM model. Currently, work is underway to revise these routines based on developments in understanding Hg complexation and uptake by methylating organisms. Under consideration are revisions that would allow the calculation of HgS^0 concentrations in sediment pore waters, and that would model HgS uptake via diffusion by methylating bacteria. Work published by Benoit et al., in 2001 showed that calculated rate of diffusion of HgS^0 into cells of a Hg-methylating SRB, *D. propionicus*, was more than sufficient to support measured rates of Hg methylation in pure culture. Further, geochemical equilibrium models that include HgS^0 formation, and its uptake into methylating bacteria via passive diffusion, explain the patterns of MeHg production found in ecosystems with sulfur gradients (Benoit et al., 1999; 2001). Nonetheless, passive diffusion has only been examined in one organism. In two studies with non-methylating microorganisms, active Hg uptake seems to be a more important accumulation pathway than diffusion. Using laboratory cultures of *Selenastrum capricornutum*, *Cosmarium botrytis*, and *Schizothrix calcicola*, Moye et al., (2001) concluded that uptake of methylmercury could not be explained by the passive diffusion of a neutral methylmercury-chloride complex, as had been observed by others in marine algal species and conditions (Mason et al., 1996). Golding et al., have been studying Hg uptake by a “Hg bioreporter,” *Vibrio anguillarum*, which was been engineered to include the mer operon and a reporter gene that manufactures luciferase, a light-emitting protein, when the bacterium takes up inorganic mercury (Golding et al., 2001). Hg uptake by this organism appears to occur via facilitated transport.

There is ongoing scientific debate about the pathways by which mercury enters cells, for example passive versus active uptake. Some efforts to model algal uptake of MeHg to match observations in lake environments the MCM models has suggested active rather than passive transport as the dominant mechanism (Hudson et al. 1994; R. Harris, Tetra Tech, Inc., personal communication). It should be noted that the relative importance of active and passive transport could be very different for bacteria taking up Hg(II) in anaerobic conditions versus algae taking up methylmercury in surface waters. Differing cell sizes, water chemistry and mercury forms would all have an influence on the rates of active and passive uptake.

ACME MESOCOSMS STUDIES

As a follow-up to the ACME ecosystem-scale study of Hg biogeochemistry in the Florida Everglades, the impacts of four key biogeochemical parameters are being studied individually using in situ mesocosms. ACME identified sulfur, Hg and potentially DOC as key variables that influence MeHg production and bioaccumulation in the Everglades. Nutrients appeared to exert a lesser effect on MeHg production. Everglades restoration efforts are underway, beginning with STA construction, and including changes in flow path and rates, in water storage, and potentially

including controls on Hg emissions. These changes are intended primarily to reduce nutrient loads to the Everglades, to restore more natural flow and hydroperiod, and to provide water storage and flood control to South Florida. Stormwater treatment areas are designed to reduce phosphate loading to the northern Everglades, but to date, they do not appear to have a large impact on sulfate loading (Miles and Fink, 1998). However, other restoration efforts that change the timing, path and rate of flow may substantially alter delivery of nutrient, sulfur and Hg to the Everglades.

In order to model the potential effects of Everglades restoration efforts on Hg cycling, the relationships between sulfur, nutrient and Hg loading and MeHg production and bioaccumulation need to be better, and separately, quantified. Sulfur, nutrients and DOC generally co-vary across the ecosystem because they arise from similar sources, making it difficult to quantify the individual relationships between each variable and MeHg production and bioaccumulation.

In 2000, ACME began a series of experiments to better quantify these individual relationships, and the interactions among these key parameters, through amendments of Hg, S, DOC and nutrients, individually and in combination, to in situ mesocosms. Although ACME has conducted short-term addition experiment to examine these relationships, they may not predict long-term responses for a number of reasons. Response of plant growth to nutrients is the obvious example, but other changes, like changes in Hg speciation and bioavailability over time, or development of microbial communities, are also important. An understanding of the relationship between Hg, S, DOC or nutrient loading and MeHg production and bioaccumulation requires a long-term, large scale approach because there are many steps between the entry of inorganic Hg to the ecosystem, its conversion to the methylated form, and bioaccumulation in fish.

ACME Phase-II mesocosms studies began with Hg dose-response studies at four sites across the Everglades. Mercury loading experiments were conducted in replicate in 1m diameter enclosures at in WCA-2A, 2B and 3A. In May and September of 2000, 3 mesocosms at each site were dosed with a Hg stable isotope. Use of Hg stable isotopes has allowed determination of MeHg production and accumulation from the new stable isotope Hg spike separately from the existing Hg pools. For comparison, short-term methylation rates were also assessed using a different isotope. Using ICP-MS, methylation of Hg stable isotopes added at levels of about 5 percent of the existing pool in sediments was detectable.

The preliminary results from Phase 2 mesocosm studies indicate that inorganic mercury dosed to the water's surface was rapidly deposited in the unconsolidated detritus layer atop the consolidated peat soil with a half-life on the order of one or two days. Methylmercury produced from the stable isotope spike began to appear within 24 to 48 hours of dosing. Most methylmercury production occurred within three days of dosing. MeHg produced from the spike accumulated rapidly in mosquitofish (*Gambusia holbrooki*), with maxima probably a few weeks after the spike. The increase in MeHg in surface sediments and in fish showed a linear response to the Hg dose. However, the slope of the response was highly variable among sites. Sites that support high levels of *in situ* MeHg production and bioaccumulation were also most sensitive to Hg additions.

The extremely rapid response in MeHg production to the Hg spikes suggests that newly deposited Hg is much more available for methylation and bioaccumulation than is existing Hg in surface soils. However, the bioavailability of "new" Hg for methylation and bioaccumulation decreased rapidly through time.

During 2001 and 2002, sulfate, Hg and DOC additions will be made to the ACME mesocosms, with a focus on responses to sulfate additions at low-sulfate sites. In late 2000, a

fifth set of mesocosms was emplaced in LNWR, in order to provide a very low sulfate site at which to conduct these experiments. The LNWR site also provides a contrast in calcium and DOC concentrations with the intermediate-level sulfate site at 3A15 in WCA-3A.

Hg-DOC complexation. Understanding and modeling Hg complexation chemistry is a key to modeling bioavailability. A number of efforts are underway to understand Hg and MeHg complexation with dissolved organic carbon, including interaction between Hg, DOC and solid surfaces particularly cinnabar and iron oxides.

Dissolved organic matter (DOM) has been implicated as an important complexing agent for Hg that can affect its mobility and bioavailability in aquatic ecosystems. However, binding constants for natural Hg-DOM complexes are not well known. Benoit et al., (in press) employed a competitive ligand approach to estimate conditional stability constants for Hg complexes with DOM isolates collected from Florida Everglades surface waters. The isolates examined were the hydrophobic fraction of DOM from a eutrophic, sulfidic site (F1-HPoA), and the hydrophilic fraction from an oligotrophic, low sulfide site (2BS-HPiA). Experimental determinations utilized overall octanol-water partitioning coefficients (D_{ow}) for ^{203}Hg at 0.01 M chloride and across pH and DOM concentration gradients. Use of this radioisotope allowed rapid determinations of Hg concentrations in both water and octanol phases without problems of matrix interference.

Conditional stability constants ($I=0.06$, 23°C) were: $\log K' = 11.9$ for F1-HPoA and $\log K' = 10.6$ for 2BS-HPiA. These are similar to previously published stability constants for Hg binding to low molecular weight thiols. Further, F1-HPoA showed a pH-dependent decline in D_{ow} that was consistent with models of Hg complexation with thiol groups as the dominant Hg binding sites in DOM. These experiments demonstrate that the DOM isolates are stronger ligands for Hg than chloride ion or EDTA. Speciation calculations indicate that at the DOM concentrations frequently measured in Everglades, 20-40 μM , significant complexation of Hg by DOM would be expected in aerobic (sulfide-free) surface waters. However, even nanomolar sulfide concentrations would be expected to out-compete DOM for Hg binding at any realistic (≤ 1 mM) DOM concentration; for instance, through the reaction $\text{Hg}^{2+} + \text{HS}^- \rightarrow \text{HgS}_{(aq)} + \text{H}^+$ ($\log K = 26.5$; Benoit et al., 1999a).

These results are consistent with the dominance of Hg-sulfide complexes in pore waters in the Everglades, as predicted in the model previously put forward in Benoit et al. (1999a). However, it should be noted that interactions other than simple ligand exchange may influence the reactivity of natural DOM toward Hg in sulfidic environments. For example, Ravichandran et al. (1998, 1999) showed that F1-HPoA enhanced dissolution and inhibited precipitation of $\text{HgS}_{(s)}$ to a greater degree (by an order of magnitude) than either 2BS-HPiA or thioglycolate, although our work suggests that the Hg complexes with these ligands have similar stability constants. Therefore, the ligand exchange reactions considered for the aerobic Hg-Cl-DOM chemical system may not adequately reflect the reactivity of DOM in the anaerobic Hg-sulfide-DOM system. Furthermore, a chemical equilibrium approach does not address how the kinetics of Hg-S-DOM interactions may affect the complexation of Hg in sediments with dynamic sulfide and DOM pools.

Miller and Mason (2001) are examining inorganic mercury binding to ferric oxide-hydroxide precipitate surfaces. It has been suggested that Fe redox cycling influences the distribution of Hg and MeHg between solid and aqueous phases in aquatic environments, in particular that Fe(III) oxyhydroxides at the sediment-water interface may block diffusive Hg and MeHg efflux from sediments through stronger complexation than occurs with reduced Fe complexes in anoxic sediment below. To examine the strength of Fe(II)/mercury interactions, binding experiments were conducted in which Hg and MeHg were mixed with freshly synthesized ferric oxide, with or

without the presence of DOM. The DOM isolates generated by George Aiken, and used above by Benoit were also used in this work. This work showed that Hg(II) forms bonds with two Fe oxide surface hydroxyl groups through two reactions; while the binding of MeHg to Fe oxide occurs through two different mechanisms. The binding of Hg with Fe oxide was greater than the binding of MeHg. However, in the presence of DOM, the binding of Hg and MeHg to Fe oxide is controlled by the interaction of the DOM with the Fe oxide surface, not the direct binding of Hg or MeHg to ferric oxide. At low concentrations, DOC enhanced the binding MeHg to Fe oxide through the formation of ternary complexes, however, in general DOC acted as a competing ligand for Hg and MeHg and reduced their binding to Fe oxide.

With regard to Hg and MeHg efflux from sediments, this new information suggests that binding of Hg and MeHg to the iron oxyhydroxide layer at the surface of many sediments is generally less strong than has been modeled in the past, because DOC generally acts to reduce Hg and MeHg binding to the oxidized Fe surfaces. Nevertheless, reduction of iron oxyhydroxides in the surface layers of sediments would result in the release of Hg and MeHg through the dissolution of the solid iron phase – whether Hg and MeHg are released as DOC complexes or otherwise. Aiken et al. are continuing their studies of the influence of DOC on cinnabar dissolution (Ravichandran 1998, 1999).

Studies on the influence of sulfate and iron using sediment slurries (Mark Marvin-DiPasquale). Marvin-DiPasquale and co-workers at the USGS-Menlo Park examined the effects of iron, sulfate and sulfide on net methylmercury production using Everglades soil homogenates dosed with radioactive ²⁰³Hg and ¹⁴C-methylmercury. Under anaerobic conditions, they found that the methylmercury production rate increased exponentially with temperature, while demethylation rates were virtually temperature-independent. Further, the temperature sensitivity of methylmercury production varied across the chemical and ecological gradients in the Everglades (Marvin-DiPasquale et al., 2001). This suggests either that different microbial communities are involved in methylmercury production along the agricultural chemical gradients, or that strong geochemical gradients of sulfur, carbon and/or iron mediate the availability of Hg(II) to methylating bacteria differently in various regions of the system.

They also found that excess, higher concentrations of sulfate, sulfide, and ferrous sulfide (pyrite) slurry inhibited net methylmercury production. Ferrous chloride stimulated net methylmercury production at one site only, WCA-3A-15. This suggests that the influence of the iron cycle on the mercury cycle is complex and is mediated by the bacterial communities, the sulfur cycle, and soil and pore water redox potentials and chemistries.

Effect of phosphate enrichment on MeHg production (ACME-SFWMD). McCormick and others at the SFWMD conducted phosphate-enrichment mesocosms studies at four sites in the Everglades over the last two to three years (McCormick et al., 1999). These experimental systems provided the opportunity to examine the influence of phosphate on MeHg production, separately from other factors (like sulfate) that co-vary with nutrients across the Everglades. Phosphate might influence net MeHg production directly either through effects on the growth of methylating and demethylating bacteria or by affecting the complexation and therefore bioavailability of Hg. However, experiments in which phosphate was added to sediment cores suggested no direct effect of phosphate on net methylation (Gilmour et al., 2000). More likely, phosphate may indirectly effect net MeHg production through enhanced plant growth, leading to higher organic carbon supply to sediment microorganisms and possibly changed redox conditions in sediments. The organic matter supply to sediments affects microbial activity in sediments, and would control sulfate-reduction and sulfide production rates at locations where sulfate is not limiting. Further, dissolved organic carbon acts as a strong ligand for Hg (Ravichandra et al., 1999; Benoit et al., 2000) and for MeHg (Hintelmann et al., 1995; Miller et al., 2001) and may inhibit the uptake of

MeHg into biota. Nutrient effects on Hg cycling that are mediated through plant growth need to be examined over the longer term. During 2000, ACME scientists worked with Newman and others to measure MeHg concentrations in surface sediments in the mesocosms. At the time of sampling, the mesocosms were at or near steady state with respect to responses to phosphate additions. This provided the opportunity to examine any effects of enhanced plant growth on net MeHg production.

The SFWMD conducted phosphate-enrichment mesocosms experiments at four sites with a range of in situ phosphate enrichment, from moderately enriched site U3 in WCA 2A, to more pristine sites in central WCA 3A, in central LNWR and Taylor Slough in ENP. While phosphate enrichment changed plant and periphyton communities in the mesocosms significantly, phosphate enrichment changed MeHg concentrations in surface sediments by less than a factor of three at any site. Further, there was no trend across sites in the direction of any MeHg response to PO₄ loading (Gilmour et al., 2001). To put these responses in context, they should be compared with the more than a hundred-fold range in MeHg concentrations and production rates across the Everglades from eutrophic northern WCA 2A to the MeHg maxima in central WCA 3A. These in situ mesocosms studies confirm and extend smaller scale studies, showing little direct or indirect of phosphate on MeHg production and accumulation in surface sediments.

RELATED RESEARCH

The METAALICUS project is a whole-ecosystem mercury (Hg) loading experiment, whose findings will bear directly on research and management of Hg in the Everglades. METAALICUS is being carried out at the Experimental Lakes Area (ELA) in northwestern Ontario. The ELA is a unique area, set aside by the Canadian federal government, where researchers can manipulate remote lakes, and study lakes over many decades. The METAALICUS study site includes a entire catchment including uplands, wetlands and a first order drainage lake. The major questions being addressed by METAALICUS are:

- What is the relationship between the amount of Hg deposited from the atmosphere and the amount of methylmercury in fish?
- How quickly will Hg in fish respond to a change in Hg deposition?
- How bioavailable is newly deposited Hg, relative to existing Hg pools in sediments and soils?

These are key questions in making regulatory decisions about future controls on Hg emissions. The total amount of Hg stored in soils and sediments of an ecosystem is many times greater than the amount entering in annual deposition. If these “old” Hg pools are equally mobile and bioavailable as newly deposited Hg, then it will take many decades for Hg emissions controls to have any effect on Hg levels in fish.

METAALICUS uses two powerful techniques that are new to the Hg research community to address these questions; one, the use of stable Hg isotopes, and two, the manipulation of a whole watershed. By adding Hg to ecosystems as specific stable isotopes, new Hg deposition can be traced separately existing Hg pools in sediments and soils. Use of stable Hg isotopes allows the study of the relative bioavailability of Hg through time after deposition; which was not possible in the past. An understanding of the relationship between Hg loading and MeHg production and bioaccumulation requires a whole-ecosystem approach because there are many steps between the entry of inorganic Hg to the ecosystem, its conversion to the methylated form, and bioaccumulation in fish.

The METAALICUS team includes 17 principal investigators from institutions in Canada and the US, and about 60 total scientists, some of whom also conduct Hg research in the Florida Everglades. Total cost for METAALICUS over four years will be about \$10M US. Initial pilot studies were conducted in 1999 and 2000, and the full-scale experimental addition began in June 2001.

Many of the techniques and approaches being developed in METAALICUS are being developed in parallel in the Everglades. In particular, the ACME team is using Hg stable isotopes to study the timing and magnitude of Hg dose-response in Everglades mesocosms (Krabbenhof et al., 2001; Gilmour et al., 2001). Parallel modeling efforts are also being developed in both ecosystems (Harris et al., 2001).

LITERATURE CITED

- Academy of Natural Sciences. 1998. Annual Report for Estuarine Research Center, St. Leonard, MD. Contract C-7690 with the South Florida Water Management District. June.
- Ambrose, Jr., R.B. and R. Araujo. 1998. Applications of the Phase I Everglades Mercury Cycling Model. Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Athens, GA, at the Third Annual Everglades Mercury Workshop, Sheraton West Palm Beach Hotel, West Palm Beach, FL. May.
- Bates, A.L., Spiker, E.C., and Holmes, C.W. 1998. Speciation and isotopic composition of sedimentary sulfur in the Everglades, Florida, USA. *Chem. Geology* 146: 155-170.
- Bates, A.L., Orem, W.H., Harvey, J.W., and Spiker, E.C. 2001. Sulfur Geochemistry of the Florida Everglades. U.S. Geological Survey Open-File Report 01-34, 47 p.
- Bates, A.L., Orem, W.H., Harvey, J.W., and Spiker, E.C. 2002. Tracing sources of sulfur in the Florida Everglades. *J. Environ. Qual.*, in press.
- Benoit, J.M., C.C. Gilmour, R.P. Mason, and A. Heyes. 1999a. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. *Environ. Sci. Technol.* 33(6): 951-957.
- Benoit, J.M., R.P. Mason and C.C. Gilmour. 1999b. Estimation of mercury-sulfide speciation in sediment pore waters using octanol-water partitioning and its implications for availability to methylating bacteria. *J. Environ. Toxicol. Chem.* 8 (10): 2138-2141.
- Benoit, J.M., C.C. Gilmour, and R.P. Mason. 2001. The influence of sulfide on solid-phase mercury bioavailability for methylation by pure cultures of *Desulfobulbus propionicus* (1pr3). *Environ. Sci. Technol.* 35(1): 127-132.
- Benoit, J.M., R.P. Mason, C.C. Gilmour, and G. R. Aiken. 2001. Mercury binding constants for dissolved organic carbon isolates from the Florida Everglades. *Geochim. Cosmochim. Acta.* 65: 24, 4445-4451.
- Benoit, J.M., C.C. Gilmour and R.P. Mason. 2001. Aspects of the bioavailability of mercury for methylation in pure cultures of *Desulfobulbus propionicus* (1pr3). *Appl. Environ. Microbiol.* 67:51-58.
- Benoit, J.M., Mason, R.P., Gilmour, C.C. Chemical and biological controls on mercury methylation in aquatic sediments. 6th International Conference on Mercury as a Global Pollutant, Minamata, Japan, October, 2001.
- Berman, M. and R. Bartha. 1986. Control of the methylation process in a mercury-polluted aquatic sediment. *Environ. Pollution (Series B)* 11: 41-53.
- Bodaly, R.A., R.E. Hecky and R.J.P. Fudge. 1984. Increases in fish mercury levels in lakes flooded by the Churchill River diversion, northern Manitoba. *Can. J. Fish. Aquat. Sci.* 41: 682.

- Chimney, M. and Z. Mosutafa. 1999. Effectiveness and Optimization of Stormwater Treatment Areas for phosphorus Removal. Chapter 6 in Everglades Interim Report, South Florida Water Management District, West Palm Beach, FL. January.
- Cleckner, L. B., C. C. Gilmour, J. P. Hurley, and D. P. Krabbenhoft. 1999. Mercury methylation in periphyton of the Florida Everglades. *Limnol. Oceanogr.* 44(7): 1815-1825.
- Craig, P.J. and P.D. Bartlett. 1978. The role of hydrogen sulphide in environmental transport of mercury. *Nature*. 275: 635-637.
- DEP. 2002. Integrating Atmospheric Mercury Deposition with Aquatic Cycling of in the Everglades: An approach for conducting a Total Maximum Daily Load Analysis for an Atmospherically Derived Pollutant. Final Report. Fla. Dept. of Envir. Prot., 2600 Blair Stone Road, Tallahassee, Florida 32399. 225 pp., Typescript. At: <http://www.floridadep.org/labs/mercury/index.htm>
- D'Itri, F.M., C.S. Annett and A.W. Fast. 1971. Comparison of mercury levels in an oligotrophic and eutrophic Lake. *J. Mar. Technol. Soc.* 5(6): 10-14.
- Dmytriw, A. Mucci, M. Lucotte and P. Pichet. 1995. The partitioning of mercury in the solid components of dry and flooded forest soils and sediments from a hydroelectric reservoir, Quebec (Canada). *Water, Air and Soil Poll.*: 1099-1103.
- Fink, L.E. 2000. ENR Project Mercury Studies: 1994-1999. Appendix 7-5. Everglades Consolidated Report. South Florida Water Management District, West Palm Beach, FL. January. 2002 Everglades Consolidated Report Appendix 2-6 09/05/01 App. 2-6-11
- Fink, L.E. and P. Rawlik. 2000. Chapter 7: The Everglades Mercury Problem in the Everglades Consolidated Report, The South Florida Water Management District, West Palm Beach, FL. January.
- Fink, L.E. 2001. The effect of surface and pore water quality on mercury bioaccumulation. Appendix 7-11 In The Everglades Consolidated Report 2001. South Florida Water Management District, West Palm Beach, FL.
- Gilmour, C. 1992. Effects of acid deposition on microbial processes in natural waters. Ch. 2 *Environmental Microbiology*, Wiley-Liss: 33-57.
- Gilmour, C.C. and E.A. Henry. 1991. Mercury methylation in aquatic systems affected by acid deposition. *Environ. Poll.* 71: 131.
- Gilmour, C.C., E.A. Henry and R. Mitchell. 1992. Sulfate stimulation of mercury methylation in sediments. *Environ. Sci. Technol.* 26(11): 2281-2287.
- Gilmour, C.C., G.S. Riedel, J.D. Coates, and D. Lovley. 1996. Mercury methylation by Iron (III) reducing bacteria. *Am. Soc. Of Microbiology 96 th General Meeting*, New Orleans, LA. May 19-23. Abstract (98) O-15: 356.
- Gilmour, C.C., G.S. Ridel, M.C. Ederington, J.T. Bell, J.M. Benoit, G.A. Gill and M.C. Stordal. 1998a. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. *Biogeochemistry*. 40: 327-345.
- Gilmour, C.C., A. Heyes, J. Benoit, G. Reidel, J.T. Bell and G.Gill. 1998b. Distribution and biogeochemical control of mercury methylation in the Florida Everglades.

- Gilmour, C.C., Krabbenhoft, D.P., Hurley, J.P., Orem, W.H., Aiken, G., Marvin-DiPasquale, M., Rawlik, P., Heyes, H., Cleckner, L.B., and Benoit J.M. Aquatic cycling of mercury in the Florida Everglades: results from four years of the ACME project. 6th International Conference on Mercury as a Global Pollutant, Minamata, Japan, October, 2001.
- Gilmour, C.C., Heyes, A., Mason, R., Miller, C. and Krabbenhoft, D.P. Use of stable Hg isotopes to assess the bioavailability of "old" vs. "new" mercury for methylation in the METAALICUS project . 6th International Conference on Mercury as a Global Pollutant, Minamata, Japan, October, 2001.
- Gilmour, C.C., A. Heyes, R.P. Mason, and J. W.M. Rudd. 2001. Response of methylmercury production and accumulation to changes in Hg loading: a whole-ecosystem mercury loading study. Presentation at the Workshop on the Fate,
- Gilmour, C.C., Heyes, A., Mason, R., Miller, C. and Krabbenhoft, D.P. 2001. Use of stable Hg isotopes to assess the bioavailability of "old" vs "new" mercury for methylation in the METAALICUS project . 6th International Conference on Mercury as a Global Pollutant, Minamata, Japan, October, 2001.
- Gilmour, C.C., A. Heyes, R. P. Mason and J.W.M. Rudd. 2001. Response of methylmercury production and accumulation to changes in Hg loading: A whole-ecosystem mercury loading study. EPA Workshop on the Fate, Transport, and Transformation of Mercury in Aquatic and Terrestrial Environments, West Palm Beach, FL, May 2001.
- Gilmour, C.C. et al., 2000. Aquatic cycling of mercury in the Florida Everglades: Results from four years of the ACME project. Platform presentation, SETAC, November 12-16, 2000. Transport, and Transformation of Mercury in Aquatic and Terrestrial Ecosystems, sponsored by the U.S. Environmental Protection Agency, the U.S. Geological Survey, and the South Florida Mercury Science Program. May 8-10. Sheraton West Palm Beach, West Palm Beach, FL.
- Golding, G., C.A. Kelly, R. Sparling, P.C. Loewen, J. W.M. Rudd, and T Barkay. 2001. Presentation at the Workshop on the Fate, Transport, and Transformation of Mercury in Aquatic and Terrestrial Ecosystems, sponsored by the U.S. Environmental Protection Agency, the U.S. Geological Survey, and the South Florida Mercury Science Program. May 8-10. Sheraton West Palm Beach, West Palm Beach, FL.
- Hurley, J.P., D.P. Krabbenhoft, L.B. Cleckner, M.L. Olson, G. Aiken and P.J. Rawlik, Jr. 1998. System controls on aqueous mercury distribution in the northern Everglades, Biogeochemistry 40: 293-311.
- Gill, G. A., N. S. Bloom, S. Cappellino, C. T. Driscoll, C. Dobbs, L. McShea, R. Mason, and J. Rudd (1999). Sediment-Water Fluxes of Mercury in Lavaca Bay, Texas. Environ. Sci. and Technol., 33: 663-669.
- Guentzel, J. 1997. The atmospheric sources, transport and deposition of mercury in Florida. Ph.D. Thesis, Florida State University, Tallahassee, Florida, USA. 177pp.
- Guentzel, J. L., W. M. Landing, G. A. Gill and C. D. Pollman (2001). Processes influencing rainfall deposition of mercury in Florida: The FAMS Project (1992-1996). Environ. Sci. and Technol., 35: 863-873.
- Gun, J., A. Goifman, I. Shkrob, J. Kamysny, B. Ginzburg, O. Hadas, I. Dor, A.D. Modestov, and O. lev. 2001. Formation of polysulfides in an oxygen rich freshwater lake and their

- role in the production of volatile sulfur compounds in aquatic systems. *Environ. Sci. Technol.* 34(22): 4741-4746.
- Harris, R.C. and others. 2001. METAALICUS: A study to determine the relationship between mercury deposition and MeHg concentrations in fish. Presentation at the Workshop on the Fate, Transport, and Transformation of Mercury in Aquatic and Terrestrial Ecosystems, sponsored by the U.S. Environmental Protection Agency, the U.S. Geological Survey, and the South Florida Mercury Science Program. May 8-10. Sheraton West Palm Beach, West Palm Beach, FL
- Hintelmann, H., P.M. Welbourn, and R.D. Evans. 1995. Binding of methylmercury compounds by humic and fulvic acids. *Water, Air and Soil Poll.* 80: 1031-1034.
- Howard, D. 1993. Trent University. Peterborough, Ontario, Canada. Personal communication. 20 July.
- Hudson, R.J.M., S.A. Gherini, C.J. Watras, and D.B. Porcella (1994) Modeling the Biogeochemical Cycle of Mercury in Lakes: The Mercury Cycling Model (MCM) and its Application to the MTL Study Lakes. In "Mercury as a Global Pollutant". Watras C.J. and J.W. Huckabee (Eds.). Lewis Publishers. pp 473-523.
- Hurley, J.P., D.P. Krabbenhoft, L.B. Cleckner, M.L. Olson, G. Aiken and P.J. Rawlik, Jr. 1998. System controls on aqueous mercury distribution in the northern Everglades, *Biogeochemistry* 40: 293-311.
- Hurley, J.P., L.B. Cleckner, and P. Gorski. 1999. Everglades Nutrient Removal Project Mosquitofish Bioaccumulation Study. Draft Report. Prepared for the South Florida water Management District, West Palm Beach, FL. Contract (PC C-8691-0300). University of Wisconsin Water Chemistry Program, Madison, WI. May.
- Jay, J.A., F.M.M. Morel, and H.F. Hemond. 2000. Mercury speciation in the presence of polysulfides. *Environ. Sci. Technol.* 34(11): 2196-2200.
- Jensen, S. and A. Jernelov. 1969. Biological methylation of mercury in aquatic ecosystems. Nature. 223: 753-754.
- Kelly, C.A., J.W.M. Rudd, R.A. Bodaly, N.P. Roulet, V.L. St. Louis, A. Heyes, T.R. Moore, S. Schiff, R. Aravena, K.J. Scott, B. Dyck, R. Harris, B. Warner, and G. Edwards. 1997. Increases in fluxes of greenhouse gases and methyl mercury following flooding of an experimental reservoir. *Environ. Sci. Technol.* 31(5): 1334-1344.
- Krabbenhoft, D.P., J.P. Hurley, M.L. Olson and L.B. Cleckner. 1998. Diel variability of mercury phase and species distributions in the Florida Everglades. *Biogeochemistry* 40: 311-325.
- Krabbenhoft, D.P., L.E. Fink, M.L. Olson, and P.S. Rawlik, II. 2000. The effect of dry down and natural fires on mercury methylation in the Florida Everglades. Conference Proceedings, International Conference on Heavy Metals in the Environment, University of Michigan, Ann Arbor. August.
- Krabbenhoft, D.P., C.C. Gilmour, W.H. Orem, G. Aiken, M.L. Olson, J.F. DeWild, S.D. Olund, A. Heyes, G.S. Riedel, J.T. Vbell, H. Lerch, J.M. Benoit, and S. Newman. 2001. Interfacing Process-Level Research and Ecosystem-Level Management Questions: Aquatic Cycling of Mercury in the Everglades (ACME) Phase II. Workshop on the Fate,

- Transport, and Transformation of Mercury in Aquatic and Terrestrial Environments. Sheraton West Palm Beach, West Palm Beach, Florida.
- Krabbenhoft, D.P., Gilmour, C.C., Orem, W.H., Aiken, G., Benoit, J.M. and Newman, S. Impact of drying, nutrients, sulfur and hg loading on methylmercury production and accumulation in the Florida Everglades: ACME phase II. 6th International Conference on Mercury as a Global Pollutant, Minamata, Japan, October, 2001.
- Krabbenhoft, D., J. Hurley, G. Aiken, C. Gilmour, M. Marvin-DiPasquale, W. Orem and R. Harris. 2000. Mercury cycling in the Florida Everglades: A mechanistic study. *Verh. Internat. Verein. Limnol.* 27:1657-1660.
- Krabbenhoft, D.P. and L.E. Fink. 2001. The effect of dry down and natural fires on mercury methylation in the Florida Everglades. Appendix 7-8 in Everglades Consolidated Report, South Florida Water Management District, West Palm Beach, FL. January.
- Krabbenhoft, D.P., C.C. Gilmour, W.H. Orem, G. Aiken, M.L. Olson, J.F. DeWild, S.D. Olund, A. Heyes, G.S. Riedel, J.T. Bell, H. Lerch, J.M. Benoit, and S. Newman. 2001. Presentation at the Workshop on the Fate, Transport, and Transformation of Mercury in Aquatic and Terrestrial Ecosystems, sponsored by the U.S. Environmental Protection Agency, the U.S. Geological Survey, and the South Florida Mercury Science Program. May 8-10. Sheraton West Palm Beach, West Palm Beach, FL
- Lamers, L.P.M., H.B.M. Tomassen, and I. G.M. Roelofs. 1998. Sulfate induced eutrophication and phytotoxicity in freshwater wetlands. *Environ. Sci. Technol.* 32(2): 199-205.
- Lange, T. 2002. Personal Communication. Re: Fish consumption Brochure (Mercury Brochure). 2pp, typescript. Florida Fish and Wildlife Conservation Commission, Eustis Fisheries Laboratory.
- Lindberg, S.E., H. Zhang and Meyers, T.P. 1999. Application of field methods and models to quantify mercury emissions from wetlands at the Everglades Nutrient Removal Project (ENR). Prepared by Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN and National Oceanic and Atmospheric Administration, ATDD, Oak Ridge, TN. Second Final Report. Everglades Mercury Air/Surface Exchange Study (E-MASE). Prepared for South Florida Water Management District, West Palm Beach, FL (C-6660). January.
- Lindberg, S.E. and H. Zhang. 2000. Air/water exchange of mercury in the Everglades II: measuring and modeling evasion of mercury from surface waters in the Everglades Nutrient Removal Project. *Science of Total Environ.* 259: 135-143.
- Leo, A., C. Hansch, and D. Elkins. 1971. Partition coefficients and their uses. *Chem. Rev.* 71: 525-621.
- Lockwood, R.A. and K.Y. Chen. 1974. Adsorption of Hg(II) by ferric hydroxide. *Environ. Lett.* 6(3): 151-166.
- Lyman, W.J., W. F. Reehl, and D.H. Rosenblatt. 1996. Handbook of Chemical Property estimation Methods. American Chemical Society. Washington, DC.

- Marvin-DiPasquale, M. C. and D.G. Capone. 1998. Benthic sulfate reduction along the Chesapeake Bay central channel. I. Spatial trends and controls. *Marine Ecology Progress Series*, 168: 213-228.
- Marvin-DiPasquale, M.C. and R.S. Oremland. 1998. Bacterial methylmercury degradation in Florida Everglades peat sediment. *Environ. Sci. Technol.*, 32(17): 2556-2563.
- Marvin-DiPasquale, M.M., J. Agee, R.S. Oremland, M. Thomas, D.P. Krabbenhoft, and C. G. Gilmour. 2000. Methyl-mercury degradation pathways: a comparison among three mercury-impacted ecosystems. *Environ. Sci. Technol.*, 34: 4908-4916.
- Marvin-DiPasquale, M.M., J. Agee and R.S. Oremland. 2001. Environmental controls on methylmercury production and degradation by bacteria in Florida Everglades sediments. Draft report to the South Florida Water Management District under contract C-11719 by U.S. Geological Survey, Menlo Park, CA. April.
- Mason, R.P., C. Miller, J.M. Benoit, A. Heyes, and C.C. Gilmour. 2001. Factors controlling methylmercury production in sediments and fate in aquatic systems. Presentation at the Workshop on the Fate, Transport, and Transformation of Mercury in Aquatic and Terrestrial Ecosystems, sponsored by the U.S. Environmental Protection Agency, the U.S. Geological Survey, and the South Florida Mercury Science Program. May 8-10. Sheraton West Palm Beach, West Palm Beach, FL.
- Mauro, J. B. N., and J. R. D. Guimaraes. 1999. Hg methylation potential in aquatic macrophytes of the Everglades Nutrient Removal Area. Report to the South Florida Water Management District, W. Palm Beach
- McCormick, P. V., P. S. Rawlik, K. Lurding, E. P. Smith, and F. H. Sklar. 1996. Periphyton-water quality relationships along a nutrient gradient in the northern Florida Everglades. *J. N. Am. Benthol., Soc.* 15 (4): 433-449.
- McCormick, P. V., R. B. E. Shuford III, J. G. Backus, and W. C. Kennedy. 1998. Spatial and seasonal patterns of periphyton biomass and productivity in the northern Everglades, Florida, U.S.A. *Hydrobiologia*, 362: 185-208.
- McCormick, P.M., S. Newman, S.L. Miao, R. Reddy, D. Gawlik, C. Fitz, T. Fontaine, and D. Marley. 1999. Chapter 3: "Ecological Needs of the Everglades" in *The Everglades Interim Report to the Florida Legislature*, South Florida Water Management District, West Palm Beach, FL.
- Miles, C.J. and L.E. Fink. 1998. Monitoring and mass budget for mercury in the Everglades Nutrient Removal Project. *Archives of Environ. Contam. and Toxicol.*, 35(4): 549-557.
- Miles, C.J. and A.. Moyer. 2000. Partitioning of methylmercury into freshwater algae. Report to the Florida Dept. of Env. Protection, Tallahassee, from the Food and Environmental Toxicology Laboratory, University of Florida, Gainesville, FL.
- Miller, C. and R.P. Mason. Effect of dissolved organic matter on the binding of mercury and methylmercury to amorphous iron oxide. American Chemical Society. Chicago, August 26 through 30, 2001.
- Moyer, A., C. Miles, and K. Merritt. 2001. Algal uptake of methylmercury. Pre-Workshop on the Fate, Transport, and Transformation of Mercury in Aquatic and Terrestrial

- Ecosystems, sponsored by the South Florida Mercury Science Program. May 7. Sheraton West Palm Beach, West Palm Beach, FL.
- Newman, S. and K. Pietro. 2001. Phosphorus storage and release in response to flooding: implications for Everglades stormwater treatment area. *Ecological Engineering*. In Press.
- Olson, B.H. and R.C. Cooper. 1976. Comparison of aerobic and anaerobic methylation of mercuric chloride by San Francisco Bay sediments. *10*: 113-116.
- Orem, W.H., Bates, A.L., Lerch, H.E., Holmes, C.W., Harvey, J.W., Corum, M., Chrisinger, M., Marot, M., and Kleckner, S. 2002. Sulfur geochemistry of the Everglades. In: *Mercury Geochemistry of the Everglades, Results of ACME Phase I* (D.P. Krabbenhoft, ed.). U.S. Geological Survey Bulletin, in review.
- Oremland, R.S., C.W. Culbertson, and M.R. Winfrey. 1991. Methylmercury decomposition in sediments and bacterial cultures: involvement of methanogens and sulfate reducers in oxidative demethylation. *Appl. Environ. Microbiol.*, 57(1): 130-137.
- Pollman, C.D., G.A. Gill, W.M. Landing, J.L. Guentzel, D. Bare, D. Porcella, E. Zillioux, and T. Atkeson. 1995. Overview of the Florida Atmospheric Mercury Study (FAMS). *Water, Air & Soil Poll.*, 80: 285-290.
- PTI. 1994. The Influence of Phosphorus on Mercury Cycling and Bioaccumulation in the Everglades. Draft. Prepared for Sugar Cane Growers Cooperative. PTI Environmental Services, Waltham, MA. March.
- Pak, K. and R. Bartha. 1998. Products of mercury demethylation by sulfidogens and methanogens. *Bull. Environ. Contam. Toxicol.*, 61: 690-694.
- Paterson, M.J., J.W.M. Rudd, and V. St. Louis. 1998. Increases in total and methylmercury in zooplankton following flooding of a peatland reservoir. *Environ. Sci. Technol.*, 32(24): 3868-3874.
- Pollman, C., G. Gill, W. Landing, D. Bare, D. Porcella, E. Zillioux, and T. Atkeson. 1995. Overview of the Florida atmospheric mercury study (FAMS). *Water, Air and Soil Poll.*, 80: 285-290.
- Ravichandran, M., G.R. Aiken, M.M Reddy and J.N. Ryan. 1998. Enhanced dissolution of cinnabar (mercuric sulfide) by dissolved organic matter isolated from the Florida Everglades. *Environ. Sci. Technol.*, 32: 3205-3311.
- Ravichandran, M. 1999. Interactions between mercury and dissolved organic matter in the Florida Everglades. Ph.D. Thesis. University of Colorado. Spring.
- Rodgers, D.W., M. Dickman and X. Han. 1995. Stories from old reservoirs: sediment Hg and Hg methylation in Ontario hydroelectric developments. *Water, Air and Soil Poll.*, 80: 829-839.
- Rawlik, P. 2001a. Mercury concentrations in mosquitofish from treatment wetlands in the northern Everglades. Appendix 7-15 In *The Everglades Consolidated Report 2001*. South Florida Water Management District, West Palm Beach, FL.
- Rawlik, P. 2001b. Stormwater Treatment Area 1 West: results of startup mercury monitoring. Appendix 7-14 in *2001 Everglades Consolidated Report*. South Florida Water Management District, West Palm Beach, FL.

- Reinfelder, J.R., and S.I. Chang. 1999. *Environ. Sci. Technol.*, 33: 1860-1863.
- Robbins, J.A., C. Holmes, J. Keeler, R. Reddy, and R. Abelac. Unpublished data. NOAA Great Lakes Environmental Laboratory, Ann Arbor, MI.
- Rumbold, D.G. 2000. Methylmercury risk to Everglades wading birds: a probabilistic ecological risk assessment. Appendix 7.3b in 2000 Everglades Consolidated Report. South Florida Water Management District, West Palm Beach, FL.
- Rumbold, D.G., L. Fink, K. Laine, F. Matson, S. Niemczyk, and P. Rawlik. 2001a. Annual permit compliance monitoring report for mercury in Stormwater Treatment Areas and downstream receiving waters of the Everglades Protection Area. Appendix 7-9 in 2001 Everglades Consolidated Report. South Florida Water Management District, West Palm Beach, FL.
- Rumbold, D.G., L. Fink, K. Laine, F. Matson, S. Niemczyk, and P. Rawlik. 2001b. Stormwater Treatment Area 6 follow-up mercury studies. Appendix 7-13 In The Everglades Consolidated Report 2001. South Florida Water Management District, West Palm Beach, FL.
- SFWMD. 1995. Everglades Nutrient Removal Project: 1994 Monitoring Report. South Florida Water Management, Prepared for the Florida Department of Environmental Protection, Tallahassee, FL.
- SFWMD. 1996. Everglades Nutrient Removal Project: 1995 Monitoring Report. South Florida Water Management, Prepared for the Florida Department of Environmental Protection, Tallahassee, FL. Appendix 8B-3 2002 Everglades Consolidated Report 09/05/01 App. 8B-3-16 DRAFT
- SFWMD. 1997. Everglades Nutrient Removal Project 1996 Monitoring Report. South Florida Water Management District, West Palm Beach, FL. March.
- SFWMD. 1998. Everglades Nutrient Removal Project 1999 Monitoring Report. South Florida Water Management District, West Palm Beach, FL. March.
- SFWMD. 1999a. Everglades Nutrient Removal Project 1998 Monitoring Report. South Florida Water Management District, West Palm Beach, FL. March.
- SFWMD. 1999b. Final Report on the Effect of Best Management Practices on the Loading of Mercury Species to/from the Everglades Nutrient Removal Project: Monitoring Program (Project C-1). Submitted by the South Florida Water Management District, West Palm Beach, Florida, to the Florida Department of Environmental Protection, Tallahassee, Florida, to fulfill the requirements of a Section 319 Grant (SP335/C-6663/4) from U.S. Environmental Protection Agency Region 4, Atlanta.
- St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, K.G. Beaty, N.S. Bloom and R.J. Flett. 1994. The importance of wetlands as sources of methylmercury to boreal forest ecosystems. *Can. J. Fish. Aquatic Sci.*, 51: 1065-1076.
- St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, K.G. Beaty, R.J. Flett, and N.T. Roulet. 1996. Production and loss of methylmercury and loss of total mercury from boreal forest catchments containing different types of wetlands. *Environ. Sci. Technol.*, 30(9): 2719-2729.

- Saouter, E., M. Gillman, R. Turner, and T. Barkay. Development and field validation of a microcosm to simulate the mercury cycle in a contaminated pond. *Environ. Toxicol. Chem.*, 14(1): 69-77.
- Scruton, D.A., E.L. Petticrew, L.J. LeDrew, M.R. Anderson, U.P. Williams, B.A. Bennett and E.L. Hill. 1994. Methylmercury levels in fish tissue from three reservoir systems in insular Newfoundland, Canada. In C.J. Watras and J.W. Huckabee, *Mercury Pollution Integration and Synthesis*. Lewis Publishers, Boca Raton, FL. 441-455.
- Sellers, P., C.A. Kelly, J.W.M. Rudd, A.R. MacHutchon. 1996. Photodegradation of methylmercury in lakes. *Nature*, 380(25): 694-697.
- Tallifert, M. A.B. Bono, and G.W. Luther. III. 2000. Reactivity of freshly formed Fe(III) in synthetic solutions and (pore)waters: voltametric evidence of an aging process. *Environ. Sci. Technol.*, 34(11): 2169-2177.
- TetraTech, Inc. 2002. Mercury Cycling and Bioaccumulation in Everglades Marshes: Phase III Report. Final Report. July.
- USEPA. 2000. South Florida Ecosystem Assessment: Everglades water management, soil loss, eutrophication and habitat. USEPA Region 4 Science and Ecosystem Support Div., EAP 904-R-00-003. September 2000. 38 pp.
- Wood, J.M, F.S. Kennedy, and C.G. Rosen. 1968. Synthesis of methylmercury compounds by extracts of methanogenic bacterium. *Nature*, 220: 173-174.
- Xia, K, U.L. Skyllberg, W.F. Bleam, P.R. Bloom, E.A. Nater and P.A. Helmke. 1999. X-ray absorption spectroscopic evidence for the complexation of Hg(II) by reduced sulfur in soil humic substances. *Environ. Sci. and Technol.*, 33(5): 786-795.
- Yin, Y. H.E. Allen, and C.P. Huang. Kinetics of mercury (II) adsorption and desorption on soil. *Environ. Sci. Technol.*, 31(2): 496-503.
- Zhang, H., and S.E. Lindberg. 2000. Air/water exchange of mercury in the Everglades I: the behavior of dissolved gaseous mercury in the Everglades Nutrient Removal Project. *Science of Total Environ.* 259: 135-143. 2002 Everglades Consolidated Report Appendix 2-6 09/05/01 App. 2-6-17 DRAFT.